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Review Article

Glutathione metabolism and Parkinson disease

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A B S T R A C T

It has been established that oxidative stress, defined as the condition in which the sum of free radicals in a cell exceeds the antioxidant capacity of the cell, contributes to the pathogenesis of Parkinson disease. Glutathione is a ubiquitous thiol tripeptide that acts alone or in concert with enzymes within cells to reduce superoxide radicals, hydroxyl radicals, and peroxynitrites. In this review, we examine the synthesis, metabolism, and functional interactions of glutathione and discuss how these relate to the protection of dopaminergic neurons from oxidative damage and its therapeutic potential in Parkinson disease.

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Introduction

Neurons are among the most metabolically active cells in the body, requiring the correct balance of oxygen and glucose to maintain healthy function. However, when the metabolic balance is overwhelmed and the sum of free radicals in a cell is greater than the capacity of the cell to detoxify these substances, oxidative stress is generated. Increased oxidative stress has been shown to contribute to the etiology or progression of a number of neurodegenerative diseases because the brain uses a disproportionate amount of oxygen per volume of tissue compared to other organs [1]. When free radicals of oxygen are present within the environment of the cell, they may damage lipid membranes, interfere with DNA integrity, and interrupt cellular respiration through alterations in mitochondrial complex I [2–4]. The reduction or

Abbreviations: ABC, ATP-binding cassette transporter; ASK1, apoptosis signal-regulating kinase 1; BBB, blood–brain barrier; BSO, l-buthionine-(*S,R*)-sulfoximine; COMT, catechol-*O*-methyltransferase; DA, dopamine; DAT, dopamine transporter; DHBT-1, 7-(2-aminoethyl)-3,4-dihydro-5-hydroxy-2*H*-1,4-benzothiazine 3-carboxylic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; γGT, γ-glutamyl-*N*-transpeptidase; GCL, glutamylcysteine ligase; GPX, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione *S*-transferase; HVA, homovanillic acid; JNK, c-Jun N-terminal kinase; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MDRP, multidrug resistance protein; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NAC, *N*-acetylcysteine; OTC, 2-oxothiadolazine-4-carboxylate; PD, Parkinson disease; Pgp, P-glycoprotein; ROS, reactive oxygen species; SIN1, 3-morpholinosydnonimine; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; VMAT2, vesicular monoamine transporter 2.

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detoxification of free radicals is handled by a number of homeostatic mechanisms under normal physiological conditions.

Parkinson disease (PD)[1] is one of the neurological disorders affected by changes in oxidative balance. PD is a progressive neurodegenerative disease with noticeable outward symptoms generally appearing in the 6th decade of life. The most common phenotypes of this disorder include progressive deterioration of autonomic and motor functions and, in some cases, cognitive decline. Although the underlying etiology of Parkinson disease is not completely understood [5,6], the most common neuroanatomical pathology is the accumulation of misfolded α-synuclein into intracellular aggregates called Lewy bodies, present throughout the enteric [7,8], peripheral [9], and central nervous systems [10,11]. Progression of the disease results in the significant loss of the dopaminergic neurons situated in the midbrain substantia nigra pars compacta.

Sources of reactive oxygen species in the substantia nigra

The loss of dopaminergic neurons located in the substantia nigra pars compacta (A9) is the lesion most characteristic of Parkinson disease, although other regions of the central, peripheral, and enteric nervous systems also show considerable cell loss [12–15]. Within the CNS, it is not entirely clear why the substantia nigra is so significantly affected, although this region does have a number of characteristics that make it particularly vulnerable to oxidative stress. These factors include (but are not limited to) the presence of endogenous dopamine, iron, and neuromelanin [16–18]. Additionally, the intrinsic antioxidant defenses in this structure are more vulnerable than in other brain regions because of lower levels of glutathione (GSH) [19,20] and glutamylcysteine ligase activity [21] and higher microglial:astrocyte ratios [22,23].

Dopamine (DA), which is the most abundant neurotransmitter in the basal ganglia [24], is synthesized in the large-diameter neurons of the substantia nigra and is released from the terminals that reside within the caudate and putamen nuclei (in rodents this is called the striatum) [25]. Functionally, dopamine modulates excitatory and inhibitory synaptic transmission, ensuring smooth directed movement [26]. When released from presynaptic terminals, DA is actively taken up from the synaptic cleft through a number of monoamine transporters (i.e., dopamine active transporter (DAT)), where it is packaged into intracellular vesicles by vesicular monoamine transporters (VMATs) [27]. In the SNpc dopaminergic neurons, the predominant VMAT is VMAT2 [28,29]. When DA is produced in excess of capacity and cannot be transported into the cell through the DAT or packaged internally by VMAT, it remains in free form, in which it can be readily oxidized to DA quinone or form superoxides and hydrogen peroxide [30–32]. These superoxides may damage cell and organelle membranes, leading to cellular dysfunction.

Inside the cell, DA quinones react with the sulfhydryl groups of the free amino acid cysteine, cysteine found in glutathione, and other cysteine residues to covalently modify proteins [31,32] that cause cellular toxicity and, in some cases, cell death [30,31,33,34]. DA quinones have also been shown to react with neuromelanin to form eumelanin [35], which is present in DA neurons of the substantia nigra (SN). DA may also autoxidize to form hydroxyl radicals ($OH^{\bullet}$) [30,32,36] or, after oxidation to hydrogen peroxide, may react with iron, copper, or oxygen (O_2) to form hydroxyl radicals [37].

Iron metabolism is necessary for the function of some enzymes, including tyrosine hydroxylase (the rate-limiting enzyme in DA biosynthesis), and for overall neuronal health [38–41]. Iron is transported into cells from the bloodstream while bound to transferrin and stored intracellularly by binding to the protein ferritin [37]. Ferritin in the cytosol comprises heavy (H)- and light (L)-chain subunits. The H-subunit has ferroxidase activity, converting Fe^{2+} to Fe^{3+}, whereas the L-subunit stabilizes the complex of subunits to remain in iron storage form. The ratios of H- versus L-type subunits of ferritin vary among tissues and in various cell types within the brain. These differences can affect the interactions of iron with other cellular components and make some cell types more vulnerable to oxidative stress [37,42].

Within the CNS, the SN is the structure containing the highest level of iron [43,44]. In a reduced state, iron (Fe^{2+}) readily reacts with hydrogen peroxide to form hydroxyl radicals via the Fenton reaction [37,45]. The ratio of reduced iron (Fe^{2+}) to oxidized iron (Fe^{3+}) is approximately 1:1 in the normal SN [46,47]. However, in PD patients the ratio of reduced to oxidized iron in the SN has been reported to increase [48], in one report to 1:3 [49]; a dysregulation not found in other tissues or regions of the brain [49,50]. Because numerous studies have shown that the elevated levels of reduced iron in the SN can lead to cellular toxicity [51–54], it has been suggested that iron chelation may provide some level of neuroprotection in Parkinson disease [55–58].

The SN contains another protein that may also contribute to oxidative stress. Neuromelanin, a brown-black insoluble substance that is formed from oxidative metabolites of dopamine and norepinephrine [59,60], has been shown to interact with lipids, pesticides, other toxic compounds including paraquat [61,62], and many heavy metal ions including iron [63–65]. Of the transition metals, neuromelanin binds most tightly with iron [62,65]. Although these interactions may initially be protective [66], when this system is overwhelmed (i.e., iron is present in excess), neuromelanin may begin to catalyze the production of free radicals [67].

Glutathione: an important antioxidant in the brain

Glutathione, a ubiquitous thiol tripeptide, provides protection from oxidative stress-induced damage through the reduction of reactive oxygen species (ROS). GSH acts alone or in concert with other enzymes to reduce superoxide radicals, hydroxyl radicals, and peroxynitrites [3]. Additionally, GSH detoxifies xenobiotics, is a storage and transfer form for cysteine, and maintains cellular redox potential by keeping sulfhydryl proteins in a reduced state [68]. The antioxidant characteristics of GSH have been demonstrated in a number of models of oxidative stress including depletion of GSH with L-buthionine-(*S,R*)-sulfoximine (BSO) [69–73] or ethacrynic acid [74] or reduction of GSH synthesis using antisense directed against γ-glutamylcysteine synthetase, hereafter referred to as glutamylcysteine ligase (GCL) (see section on GSH synthesis below) [75–78], or glutaredoxin 2 [79]. In these studies, diminished levels of GSH increase oxidative stress in whole cells as well as in mitochondrial fractions and increase lipid peroxidation, intracellular calcium, and γ-glutamyl transpeptidase (γGT) activity.

Several studies discussed below illustrate these points by utilizing dopaminergic systems. Depletion of GSH by BSO, an irreversible inhibitor of GCL that does not by itself induce nigrostriatal damage in vivo [80], potentiates the amount of MPTP-induced tyrosine hydroxylase-positive (TH^{+}) neuron death in the SNpc (48.6% cell death compared to 30.1% cell death) [69,80]. Additionally, under conditions of increased oxidative stress such as when mesencephalic cells are placed in culture or during normal aging in vivo, decreasing GSH level causes neuron loss [76].

The reduction of GSH activity by ethacrynic acid (EA), an effective loop diuretic used in clinical practice [81], has also been shown to increase cell sensitivity to free radicals. Astrocytes exposed to EA and

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